Synopsis

of

D.Sc. Research work

Entitled

Studies on some aspects of application of bioinformatic tools for ascertaining systematics and phylogeny of freshwater oligochaetes

to be undertaken by

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Title

Studies on application of bioinformatic tools for ascertaining systematics and phylogeny of freshwater oligochaetes

- **STATE OF THE ART**

As estimated 50% or more of the macroinvertebrate communities in Indian lakes, rivers, and streams are often made up of oligochaetes. A significant number of Oligochaetes are found coexisting with the macrobenthic fauna in estuaries, coastal regions, and locations close to the beach. Oligochaetes are an essential part of aquatic communities worldwide, not just in freshwater but also in estuaries and the ocean. Because of the way they live, this group greatly contributes to the diet of bottom-feeding omnivores and to the system's nutritional dynamics[1]. The use of freshwater oligochaetes as bioindicators[2-4] of aquatic pollution and a helpful tool for biomonitoring [5-7], lake typology[8], and indicator of trophic state has been the subject of numerous environmental research.

Stephenson (1930)[9] published the first systematic record of the aquatic and terrestrial Oligochaetes of the Indian subcontinent in the Fauna of British India series; nevertheless, he made no reference of the Oligochaete fauna of this area of India (Jharkhand, Bihar, M.P.). A survey of the literature revealed that there are many dispersed, unorganised investigations on freshwater oligochaetes. Naidu (1965, 1966)[10-13] happens to be pioneer worker on freshwater oligochaetes of India. Later Naidu and Naidu (1979)[10] reported oligochaete fauna from Kashmir; Naidu and Srivastava (1980)[11] from Nagpur, Sinha et al. (1989; 1991; 1993)[14-16] from Jharkhand; Ragi (2014)[17] from Thiruvananthapuram. Similarly

Freshwater Oligochaetes from Western Ghats and coastal Karnataka were studied by Hegde and Shreepada (2015)[18]. Preliminary research on freshwater oligochaetes from a few areas in Tamil Nadu was conducted by Chakma et al. (2020)[19]. Singh (2009)[20], on the other hand, reviewed aquatic oligochaetes in Indian context and provided a checklist

containing 147 specimens of Oligochaetes, representing 102 species, 17 genera, and 4 families. Examining previous research makes it evident that there is a dearth of studies and information about freshwater oligochaetes in India overall, and in Jharkhand specifically. There is no specific work from Jharkhand that has been documented on taxonomy and phylogeny based on morpho-anatomical traits and DNA investigations utilising bioinformatic techniques on freshwater Oligochaetes.

When compared to the genetic configuration of the same species from different regions and different climate conditions, the investigation will shed light on the ecogenetics of the group. The distance matrix calculation will show the ecogenetic influence and indicate the differences in response to environmental factors other than medications.

- **PROBLEMS TO BE INVESTIGATED**

The current state of research on the topic amply demonstrates that the freshwater oligochaete fauna of this area, which includes Jharkhand and Bihar, has not yet been subjected to taxonomic or phylogenetical analysis. Freshwater oligochaetes from various lotic and lentic habitats in Jharkhand will be sampled, preserved, and tallied per square meter in the current proposed research project. Each specimen will undergo detailed taxonomy based on morpho-anatomical features as well as genetic features to establish species and phylogeny. The inter and intra specific variation will be found out.

Geotagging will be carried out during sampling in order to prepare a distributional pattern for the group throughout the state. We'll compute the species diversity, population density, and composition of the community.

- **OBJECTIVES**

The current study aims to obtain first hand information on the systematics and phylogeny of the state's freshwater oligochaetes, along with the first-ever distribution mapping of each species by geotagging. Establishing the phylogeny of oligochaetes based on bioinformatic properties is another goal, as it hasn't been done for aquatic oligochaete

species yet. The phylogeny so far available is primarily based on variable characters and so there is a lot of debates and controversies. The present study will provide a clear base of it. Diversity of species and the projected number of species, in addition to the actual number, will be estimated.

- **HYPOTHESIS**

The study will offer first-hand knowledge of the state's freshwater oligochaetes' biodiversity spectrum, including previously unknown genetic feature-based phylogeny and the first genuine taxonomic species.

- **RESEARCH DESIGN**

1. The freshwater oligochaete samples will be collected and preserved.
2. Community composition will be studied and population densities will be enlisted.
3. Population dynamics, species diversity, evenness, equitability based on expected number of species considering broken stick model of Mc Arthur will be analyzed.
4. After collecting, the samples will be photographed and geo-tagged.
5. DNA extraction, PCR amplification and sequencing will be done.
6. Samples will be identified and classified by analyzing the sequences using various bioinformatic tools.
7. Phylogenetic tree will be prepared, and other analyses will be done.

- **METHODOLOGY**

1. Collection: freshwater oligochaetes will be collected by scooping or dredging.
2. Preservation of the samples will be done in 70% alcohol.
3. For photography additionally geotagging will be done using appropriate handheld GPS device.
4. DNA extraction will be done; quality will be evaluated on Agarose Gel (1%).
5. PCR: Using a thermal cycler, extracted DNA will be amplified using a COI-specific primer or other appropriate marker.[17,18]

6. Sequencing: Sanger sequencing will be performed after the PCR amplicon has been bead purified[21, 22]
7. For species level confirmation, BLAST (Basic Local Alignment Search Tool) will be employed with values included in scoring system of BLAST such as Max Score, query cover, E Value, Total Score, and Identity percent. BLAST results will be expressed as a graphic overview, alignments, and MSA, etc[23].
8. Distance matrix will be prepared using Neighbor-joining method.[23]
9. Phylogenetic tree will be prepared using the tools present in the BLAST results page or other appropriate software.[19]

- **STATISTICAL ANALYSIS**

Data collected during sampling will be statistically analyzed. Species diversity will be calculated following Shannon and Wiener[24], Hurlbert[25], Simpson[26], and evenness, following Pleiou (1975)[27]. Expected number of species in a habitat will be calculated following Llyod and Ghelardi (1964)[28], which is based on broken stick model of McArthur[29] of Niche.

- **TOOLS TO BE USED**

1. Benthic collection devices like scoop, dredge.
2. Ethyl alcohol
3. Glass/Plastic containers
4. Sieve of different BS grade.
5. Centrifuge
6. Thermal cycler
7. Windows based PC and online tools
8. Software applications such as Arc-GIS, SPSS, etc.
9. Chemicals etc.

- **SIGNIFICANCE OF STUDY**

The study on freshwater oligochaetes of the state will be a first hand and novel contribution, not only to the existing scientific knowledge on molecular taxonomy and phylogeny but also original contribution to the subject. Further the results of the study will be first time record of freshwater oligochaete biodiversity on genetic level. The findings will put an end to the debates and controversies pertaining to phylogeny of concerned species as it will be based on genetic features.

- **RELEVANCE TO THE PRESENT-DAY PROBLEM AND NEED OF THE SOCIETY**

Any type of biodiversity is necessary for the continuation of human life on Earth. Biodiversity cannot be produced; instead, it can only be preserved for future human benefit by being identified both genetically and morphologically. In addition to benefiting society and demonstrating its relevance, the current work will also play a significant role in sustainable development.

- **CONTRIBUTION TO EXISTING KNOWLEDGE**

No such works has been taken in Jharkhand. The present work will provide the firsthand information on phylogeny and molecular taxonomy and ecology of freshwater oligochaetes of Jharkhand. The inter and intra specific genetic variation will also be known by it.

- **FUTURE WORK**

The outcome of the work will throw light on the distribution, phylogeny, and molecular taxonomy of freshwater oligochaetes of Jharkhand, which will be helpful in conservation related studies. The gene pool in freshwater oligochaetes may be manipulated in future for various need-based uses.

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